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Geographical variation of the essential oil components in *Asiasarum heterotropoides*

中村輝子*・遠藤次郎*・長沢元夫*: オクエゾサイシンの精油成分の地理的変異

Asiasarum heterotropoides (Fr. Schmidt) F. Maekawa is distributed in Tohoku, Hokkaido, Sakhalin and the Southern Kuriles. Nagasawa (1961) made a chemical analysis of the essential oils of the species from three different areas in Hokkaido, and discovered a remarkable quantitative variation among them. The present study was undertaken in order to clarify the compositional differences of the essential oils within the species in more detail.

Materials and Methods The plant materials used in this study are listed in Table 2. Since preliminary tests revealed no seasonal variation, materials were obtained from May to September. In all cases, fresh materials were subjected to analysis.

The subterranean parts were chopped into pieces and extracted with chloroform. The chloroform layer was evaporated under reduced pressure at around 45°C to give a fragrant pale yellow oil, which was developed on thin layer chromatography using Kieselgel G nach Stahl (Merck). The following solvent systems were employed in succession: first pure petroleum ether; and then mixtures of petroleum ether and ether in the ratios of 20:1, 4:1, 1:1 and finally 1:2. The chloroform extract was also examined with gas chromatography (Shimazu GC-20, 6 mm dia. 2.25 m, PEG 6000 and 20 M, 60-80 mesh). In the gas chromatograms, the area of each peak was calculated. The gas chromatograms and the thin layer chromatograms of all the samples were compared.

On the other hand, in order to ascertain the structure of each substance in chloroform extract, each substance was isolated by using column chromatography. The elution of column was effected by a mixture of petroleum ether

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Table 1. The essential oil components in *A. heterotropoides*.

class	substances
aliphatic hydrocarbon	*n*-pentadecane
orcinol aromatic	3, 5-dimethoxytoluene, 3, 4, 5-trimethoxytoluene, 2, 3, 5-trimethoxytoluene
phenylpropanoid	methyleugenol, elemicine, safrol, croweacine, asaricine
monoterpenoid	limonene, linalool, eucarvone, car-3-ene-2, 5-dione

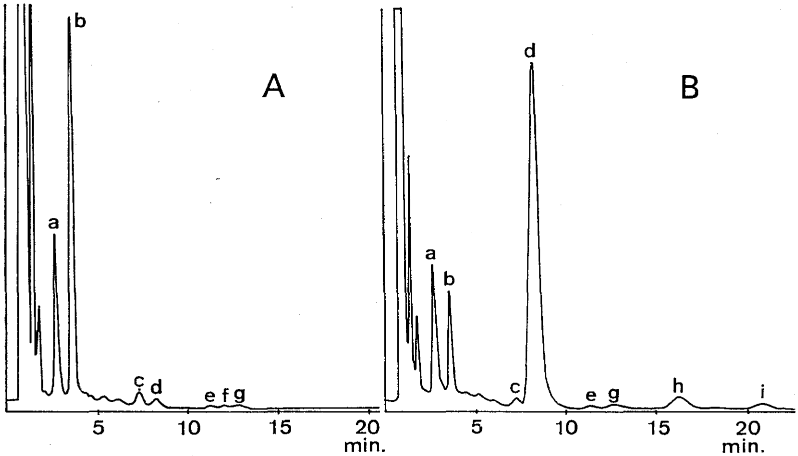

Fig. 1. Gas chromatograms of the essential oil of *A. heterotropoides*. A: Mt. Taihei, B: Bihuka, a; pentadecane, b; eucarvone, c; dimethoxytoluene, d; methyleugenol, f; carenedione, g; trimethoxytoluene, h; croweacine, i; elemicine.

and ether with increasing ether ratio. This process was repeated until each substance was individually isolated. After isolation, each substance was analyzed with a number of spectroscopies, and the structure of the substance was determined by spectroscopic evidences. Isolation was so difficult that this work was carried out on the samples from only two localities. The isolated substances were also used as standard substances in the gas chromatographic tests as well as in the thin layer chromatographic tests of the chloroform extracts mentioned above.

Results and Discussion The essential oil components in chloroform soluble portion obtained from the subterranean parts of *A. heterotropoides* are sum-

Table 2. Geographical variation of monoterpenoid and phenylpropanoid in *A. heterotropoides*.

Component / Locality	monoterpenoid	phenylpropanoid				
	eucarvone (%)	methyleugenol (%)	elemicine (%)	safrol (%)	croweacine (%)	asaricine (%)
Hokkaido						
Hamatonbetsu	97.0	3.0	—	—	—	—
Togeshita	96.0	2.0	—	2.0	—	—
Bihuka	96.0	1.0	—	3.0	—	—
Mt. Piyashiri	65.0	30.0	—	4.0	—	—
Asahikawa	93.0	6.5	—	0.5	—	—
Kamuikotan	24.0	0.6	2.4	73.0	—	—
Aizan-kei	42.0	1.0	34.0	23.0	—	—
Tennin-kyo	7.0	2.0	35.0	56.0	—	—
Mt. Rausu	57.4	34.6	1.0	7.0	—	—
Utoro	17.7	57.8	14.5	10.0	—	—
Mt. Shari	22.0	27.0	8.0	43.0	—	—
Kamisato	30.0	24.0	7.0	39.0	—	—
Onbetsu	97.0	1.5	—	1.5	—	—
Nukibetsu	35.5	26.8	—	0.5	—	37.2
Hiratori	88.2	8.0	—	3.8	—	—
Samani	57.7	3.0	4.3	35.0	—	—
Horoman	65.3	13.9	—	20.8	—	—
Moshiri	8.8	15.0	—	7.3	—	68.9
Tobetsu	97.0	2.8	—	0.2	—	—
Kotoni	93.0	3.0	—	4.0	—	—
Jozan-kei	98.0	0.5	—	1.5	—	—
Jozan-kei	24.0	3.0	—	73.0	—	—
Zenibako	18.7	0.2	2.3	78.8	—	—
Doronoki	91.0	8.8	—	0.2	—	—
Horikkapu	96.0	0.5	—	3.5	—	—
Kyowa	97.5	1.0	—	1.5	—	—

Kuromatsunai	97.0	1.5	—	1.5	—	—
Hakodate	22.0	19.5	1.5	57.0	—	—
Onuma	17.0	2.0	1.0	80.0	—	—
Aomori Pref.						
Mt. Eboshi	94.6	3.4	—	2.0	—	—
Mt. Eboshi	82.7	2.2	—	7.7	7.4	—
Mt. Hakkoda	25.5	0.2	—	66.8	7.5	—
Mt. Hakkoda	12.4	0.3	13.8	73.5	—	—
Mt. Iwaki	25.0	1.6	0.4	73.0	—	—
Akita Pref.						
Mt. Taihei	11.7	0.2	1.0	82.7	4.4	—
Mt. Komagatake	97.0	1.5	—	1.5	—	—
Mt. Komagatake	27.7	0.6	6.0	65.6	—	—
Kunimi Pass	11.0	0.2	0.8	88.0	—	—
Mt. Magusa	10.6	0.3	—	85.5	3.6	—
Iwate Pref.						
Hachimantai	23.0	5.6	4.8	66.6	—	—
Hachimantai	19.0	—	17.0	64.0	—	—
Hachimantai	22.8	1.4	1.4	71.0	3.4	—
Mt. Chausu	18.0	—	—	78.0	4.0	—
Mt. Chausu	17.3	13.2	3.5	66.0	—	—

marized in Table 1. Among them, phenylpropanoids and monoterpenoids varied qualitatively and quantitatively depending upon the sample localities. However, no geographical variation was recognized in *n*-pentadecane and orcinol aromatics. Therefore, geographical variation of amounts of monoterpenoids and phenylpropanoids will be discussed in the present paper.

For the purpose of comparison, relative percentage of each substance in the sum of phenylpropanoids and monoterpenoids[1] was calculated using the gas chromatograms (Table 2).

Quantitative variation in phenylpropanoids and monoterpenoids. The percentages of phenylpropanoids in the total amount of phenylpropanoids and monoterpenoids are presented in Fig. 2 where three groups are recognized in *A. heterotropoides*. In the first group, the amount of

[1] Limonene, linalool and carenedione existed in only the tiniest traces, and consequently the percentages of them were negligible. Therefore, in the following discussion monoterpenoids include only eucarvone.

phenylpropanoids ranges more than 70% and the amount of monoterpenoids less than 30%. The second group comprises the samples in which phenylpropanoids are contained less than 30% and monoterpenoids more than 70%. The third group shows percentages intermediate between those of the former two groups.

As for the geographic distribution, the first group often occurred in the Tohoku district. The samples from Hokkaido cannot be specified to a single group and all the three groups were frequently found.

Phenylpropanoids and monoterpenoids differ greatly in their biosynthetic pathway. Phenylpropanoids are said to be induced by means of the shikimic acid pathway while monoterpenoids are said to be produced via the mevalonic acid pathway. Our study showed that either phenylpropanoids or monoterpenoids were predominated depending on the growing localities. Phenylpropanoids are considered to be basic components in the tribe Asareae (=*Asarum*, sens. lat.), while monoterpenoids are sporadically distributed among them (Fujita, 1966; Saiki et al., 1967). In addition, monoterpenoids were produced in large amount in some samples of *A. heterotropoides*. Therefore, we supposed that monoterpenoids might have become the major component in the samples along with the

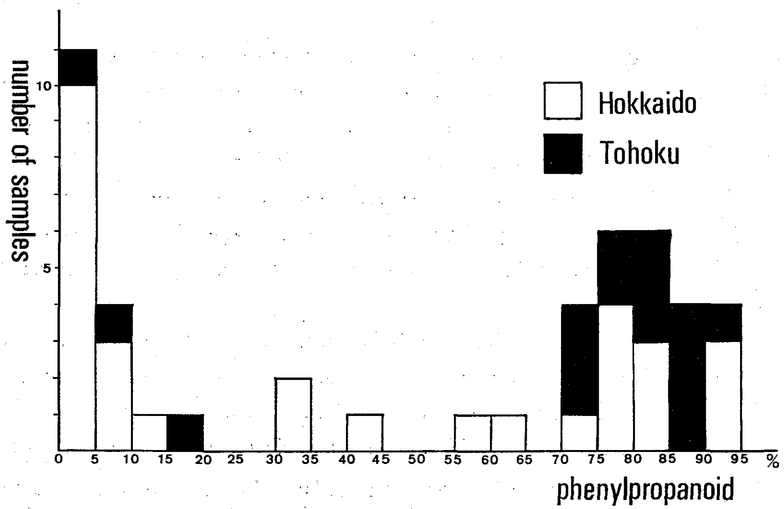

Fig. 2. Variation in the percentages of phenylpropanoids in the total amount of phenylpropanoids and monoterpenoids.

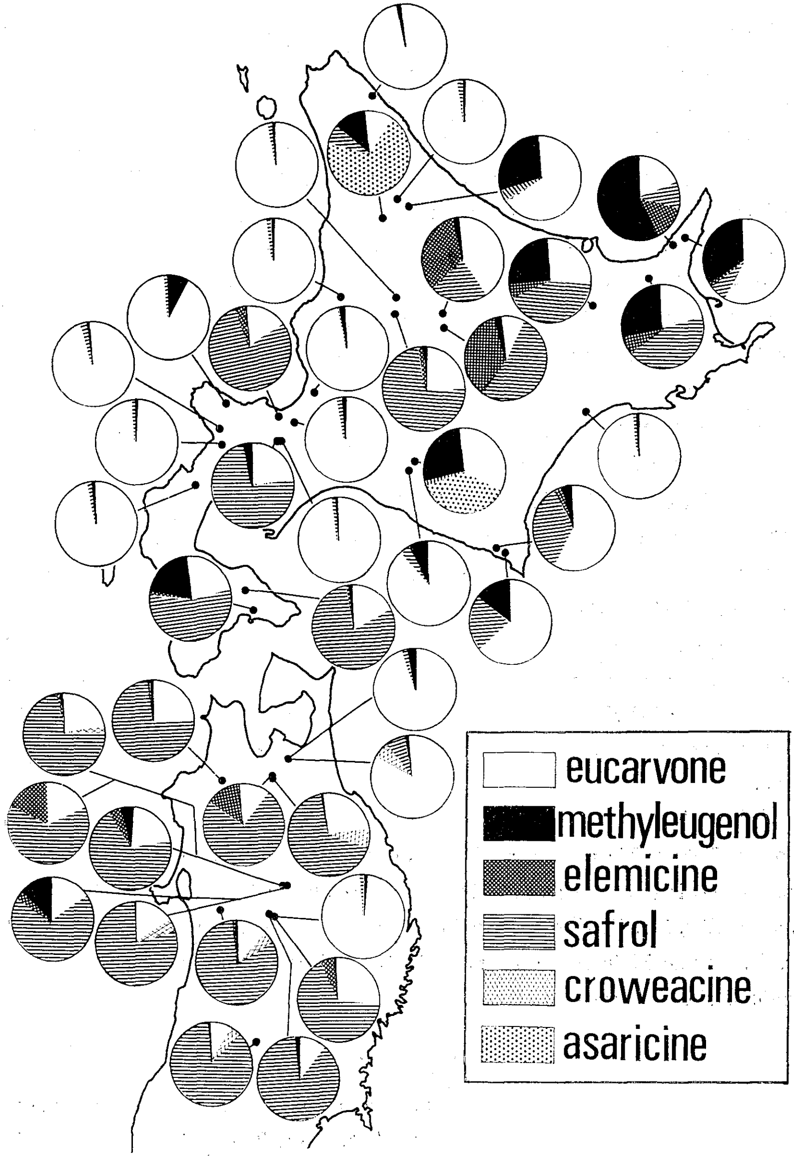

Fig. 3. Geographical variation of monoterpenoids and phenylpropanoids in *A. heterotropoides*.

speciation.

Variation in phenylpropanoids. The amount of each phenylpropanoid component varied depending on the samples. Methyleugenol and safrol had the greatest quantitative variation among them (Fig. 3). For example in a sample from Mt. Raus in Hokkaido, methyleugenol accounted for almost 69% of the phenylpropanoids content, while in a sample from Mt. Komagatake in the Tohoku region safrol constituted nearly 99% of the phenylpropanoids content. In most of the samples from Tohoku, the percentage of safrol was extremely high whereas the materials from Hokkaido contained safrol, methyleugenol, asaricine, or elemicine as the major component. Samples from Tohoku contained only traces of methyleugenol, asaricine, and elemicine

It was previously reported that phenylpropanoid compounds were extremely unstable in the amount and they were sometimes lacking (Fujita, 1966). In *A. heterotropoides* the similar variation was demonstrated. In this species clinal variation as in *A. sieboldii* (Nagasawa, 1961) was not found due to the random variation in phenylpropanoids amount (Fig. 3). However, one variation tendency common to *A. heterotropoides* and *A. sieboldii* was recognized. Both in these species the samples which contained methyleugenol as the major component occurred most often in the north-eastern parts of the distribution ranges of each species. In contrast, those samples containing safrol or any of the other oxidized derivatives such as elemicine, croweacine, or asaricine, occurred in the south-western portions of their distribution areas.

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References

Fujita, Y. 1966. Classification and phylogeny of the genus *Asarum* viewed from the constituents of essential oils. Bot. Mag. Tokyo **79**: 783-790. Nagasawa, M. 1961. Analysis of Radix Asiasari by infrared spectrophotometry. Yakugaku Zasshi **81**: 129-138. Saiki, Y., Y. Akahori, K. Morinaga, T. Taira,

T. Noro, S. Fukushima & T. Harada. 1967. The gas chromatography on the volatile oils of the plants belonged to *Heterotropa* genus (3). Yakugaku Zasshi 87: 1544-1547.

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オクエゾサイシンの地下部の精油成分の地理的変異を検討した。精油を構成するモノテルペン類とフェニルプロパン類との量的変異により，三つの群を識別できた。第1群；フェニルプロパン類が主成分であり，モノテルペン類は少ない。第2群；モノテルペン類が主成分であり，フェニルプロパン類は少ない。第3群；モノテルペン類とフェニルプロパン類との間には極端な量的差異がない。東北地方には第1群に属する検体が多く，北海道では三つの群が不規則に出現した。

質的な変異はモノテルペン類には認められず，全ての検体からユーカルボン等が検出された。一方，フェニルプロパン類の構成成分には質的および量的な変異がみられた。北海道にはメチルユーゲノールを主成分とする検体が存在したが，これは分布域の西南部の東北地方では認められなかった。東北地方の検体のほとんどのものではサフロールが主成分であった。フェニルプロパン類におけるこのような変異の傾向はウスバサイシンのそれとほぼ類似している。

□Graf, A. B.: **Tropica,** color cyclopedia of exotic plants and trees from tropics and subtropics. 1120 pp. 内カラープレート 928. 1978, V. Roehrs Co. U. S. A. ¥32,200. 同じ著者の Exotica のカラー版といったところ。ただし，少しくどい位の園芸品種の羅列のところもあった前著にくらべて，ずっと選択されすっきりとしている。しかし，色彩は少し弱いところがあるのは惜しいが，それでもどのページをあけても中々面白い。科の横文字の ABC 順，ただし食虫植物，針葉樹，シダ類，果樹はまとめられている。サボテン，ヤシ，ランなどが特に多い。Exotica よりも，見慣れぬ種類をさぐるのは便利と思う。　　(前川文夫)

□創土社編： **日本アルプスの花と蝶** 445 pp. 内プレート 416. 1979, VI. 創土社。東京。¥38,000. 花と蝶とうたってあるが，大部分は高山植物である。大場達之，河野昭一，里見信生，清水建美，高橋秀男，豊国秀夫，鳴橋直弘，橋本竹二郎の八氏が高山植物を分担している。キク科からはじめ，針葉樹に終り，シダはふくめてないが，主に一種一ページに割りつけ，カラー写真を主として記述を附記してある。大版でしかもひろくとったので花のディテールがよくわかるし，ミスズランやコハクランなどの珍らしいものも載っている。高山帯の景観や高山蝶9種の生活史も見られるので，少し高いが一本を備えるとよいだろう。末尾に豊国・清水両氏の日本アルプスの高山植物が載っている。　　(前川文夫)